

HEAT SHOCK PROTEIN 90 GENES OF TWO SPECIES OF POULTRY *EIMERIA*: EXPRESSION AND EVOLUTIONARY ANALYSIS

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ABSTRACT: Heat shock protein 90 (Hsp90) is 1 of the most abundant and evolutionarily conserved proteins. In most species, Hsp90 is essential for proper cell function. In this study, we present the molecular analysis of Hsp90 from *Eimeria* species, the causative agents of avian coccidiosis. The full-length *Eimeria acervulina* Hsp90 complementary DNA was isolated from intestinal intraepithelial lymphocytes of *Eimeria*-infected chickens. From evolutionary analysis and sequence identity, it is likely that *Eimeria* Hsp90 sequences described thus far encode the cytosolic versions of the protein. Although at the nucleotide and amino acid levels *Eimeria tenella* and *E. acervulina* Hsp90 are highly similar, their expression profiles differ considerably. Although *E. tenella* transcripts were detected in all developmental stages tested, *E. acervulina* transcripts were not found in oocysts undergoing sporulation or in fully sporulated oocysts, suggesting that messenger RNA expression may be regulated quite differently between *Eimeria* species.

Coccidiosis in chickens is caused by 7 species of apicomplexan protozoa belonging to *Eimeria*. The infection results in the destruction of the intestinal epithelium leading to diarrhea, weight loss, and anorexia (Allen and Fetterer, 2002). As is characteristic of apicomplexans, the life cycles of *Eimeria* spp. are complex and the developmental stages are distinct from one another. Sporozoites invade the intestinal epithelia, followed by several rounds of asexual reproduction characterized by the presence of schizonts and merozoites. Subsequently, macro- and microgametes are produced, which undergo sexual reproduction resulting in the formation of unsporulated oocysts that are shed in the feces. The oocysts undergo sporulation outside of the host, and the fully sporulated and infectious oocysts containing 8 sporozoites can remain viable outside of the host for at least several months. Clearly, these parasites encounter drastically different environments, from those in the warm-blooded vertebrate host to those in the soil or bedding. Each of these environments may pose distinct requirements for the maintenance of homeostasis.

In most evolutionary lineages, heat shock proteins (Hsps) maintain homeostasis inside the cell. These molecular chaperones are usually constitutively expressed. However, their expression increases under stress, such as heat or nutrient deprivation (Lindquist and Craig, 1988). Hsps are highly conserved throughout evolution and function as molecular chaperones, thereby stabilizing proteins during assembly, transport, and denaturing stress. Although many Hsps have similar names often indicating molecular size, i.e., Hsp70, Hsp90, these molecules are, nonetheless, structurally unrelated, and each member uses a different strategy in preventing misfolding of proteins. Hsp90 is essential for survival, and it makes up to 1–2% of total cytosolic proteins found in eukaryotic cells (Borkovich et al., 1989). The major functions of Hsp90 may be to stabilize proteins that are involved in cell signaling and in de novo synthesis of proteins that have difficulties reaching their native conformations (Nathan et al., 1997).

Very little is known about Hsps of apicomplexans, although it is likely that these proteins play an essential role in these

organisms especially because they undergo many environmental stresses throughout their life cycle. Hsp90 has been previously isolated from apicomplexans that include important human parasites, such as *Plasmodium falciparum* (Bonney et al., 1994), *Toxoplasma gondii* (Ahn et al., 2003), and *Theileria parva* (Gerhards et al., 1994). To date, Hsp90 has been described in a single species of *Eimeria* that infects cattle, *Eimeria bovis* (Clark et al., 1996). Our studies focus on Hsp90 of 2 species of apicomplexans responsible for poultry coccidiosis, i.e., *Eimeria acervulina* and *Eimeria tenella*. In this study, we describe the gene encoding *E. acervulina* Hsp90, the messenger RNA (mRNA) expression profiles of Hsp90 in *E. acervulina* and *E. tenella*, and provide an evolutionary analysis of Hsp90.

MATERIALS AND METHODS

Host infection and parasite recovery

Broiler chickens (Moyer's Hatcheries Inc., Quakerstown, Pennsylvania), 4–5 wk old, were each infected with 1×10^5 to 1.25×10^5 oocysts of the Wampler isolate of *E. tenella*, 1×10^5 sporulated oocysts of isolate 12 of *E. acervulina*, or 1×10^5 oocysts of Eastern Shore isolate of *Eimeria maxima* mixed in feed. Birds were housed at Animal and Natural Resources Institute, Beltsville, Maryland, and had free access to food and water. *Eimeria tenella* oocysts were collected 7 days postinoculation (pi), as described previously by Fetterer and Barfield (2003). To recover *E. acervulina* oocysts, feces were collected over 96–106 hr pi. Feces were mixed with tap water and ground in a blender. The mixture was then passed through a coarse strainer. The remainder of the oocyst recovery was carried out as described previously (Fetterer and Barfield, 2003). After cleanup of *E. tenella* and *E. acervulina* oocysts, the sample volume was divided into 5 equal portions. Four of these were sporulated in $1 \times$ phosphate buffered saline (PBS) in the presence of antimycotics and antibiotics, as described previously (Fetterer and Barfield, 2003). The remaining one-fifth was not subjected to the sporulation treatment but was frozen in the unsporulated state. At 4 time intervals commencing after placing oocysts in $1 \times$ PBS (*E. tenella*: 12, 18, 48, and 72 hr; *E. acervulina*: 3, 7, 24, and 72 hr), an equal volume of oocysts was removed, inspected under the microscope, centrifuged, and frozen. Sporozoites were obtained from *E. acervulina* and *E. tenella* by excysting sporulated oocysts using previously published methods (Fetterer and Barfield, 2003).

To obtain *E. tenella* merozoites, 5–10, 3-wk-old broiler chicks were inoculated using gavage with 4.6×10^5 oocysts. Chickens were killed 110 hr pi and the intestinal epithelia of the cecae were scraped using a glass slide. To obtain *E. acervulina* merozoites, chicks were inoculated using gavage with 1×10^5 oocysts and were killed 89 hr pi. The intestinal epithelium of the duodenum and part of the small intestine was removed and scraped with a glass slide. The subsequent method used for merozoite extraction and purification was carried out using previously published procedures (Xie et al., 1990).

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Complementary DNA library construction from tissues of infected chickens

Three-wk-old chickens were infected with oocysts of *E. acervulina* or *E. maxima*. Chickens were reinfected at 9 wk of age. Intestinal intraepithelial lymphocytes (IELs) were isolated 4 days after the secondary infection and were pooled before RNA extraction. Total RNA was isolated using TRIzol (Invitrogen, Carlsbad, California), and poly(A)⁺ RNA was prepared using the PolyA Tract mRNA Isolation System IV (Promega, Madison, Wisconsin) and reverse transcribed using oligo(deoxythymidylic acid) primers and Superscript II reverse transcriptase (Invitrogen). The complementary DNAs (cDNAs) were cloned into the pCMV-SPORT 6 vector (Invitrogen). More than 30,000 expressed sequence tags (ESTs) were generated from this library using high throughput methods. For detailed summary of library construction and screening please see Min et al. (2003).

Polymerase chain reaction

To confirm that the full-length Hsp90 transcript was derived from *E. acervulina*, a series of polymerase chain reactions (PCRs) was carried out using *E. acervulina* genomic DNA as template. Forward primer KM45 (5' GGG AAG AGC ATT TGG CTG TGA AGC ACT TC 3'), nested forward primer KM46 (5' GGG TCA GCT CGA GTT CAA GGC TCT CCT C 3'), and reverse primer KM48 (5' CTC CTT GAA CGA GAC CAT ATC TTC TCC AG 3') were used in a seminested PCR. A second PCR using forward primer KM46 and reverse primer KM23 (5' CGC TCC ATG TTC GCT GAC CA 3') was also carried out. Each reaction included 200 ng of target DNA. Thermally activated polymerase (TAP) (CLP, San Diego, California) in the presence of 400 nM primer was used. Amplifications were carried out as follows. Initial heat activation of polymerase at 95 C for 7 min; denaturation, 94 C for 30 sec; annealing, gradient of 65 C for 30 sec; extension, 72 C for 1 min; and a final extension of 5 min at 72 C. Cycles 2–4 were repeated 30 times.

Developmental expression of Hsp90 mRNA using quantitative real-time reverse transcription–polymerase chain reaction

All tissues used in RNA isolation were snap frozen after purification and stored at –70 C until use. Total RNA was isolated from *E. tenella* and *E. acervulina*: merozoites, sporozoites, and oocysts subjected to a sporulation time course, which included samples ranging from unsporulated to fully sporulated oocysts. Each sample was combined with approximately 3 g of diethylpyrocarbonate-treated Pyrex beads (3 mm diameter, Corning, Corning, New York) and 10 ml of TRIzol (Invitrogen). Samples were vortexed for 1 min and then incubated on ice for 1 min (4 times). The remainder of the total RNA isolation protocol was carried out using the manufacturer's recommended instructions. The resulting pellets containing total RNA were resuspended in DNase-RNase-free water (Invitrogen) and stored at –70 C. All RNA samples were treated with DNase I (Invitrogen) before cDNA synthesis. cDNA was synthesized from 0.8 µg of total RNA using random hexamer primers with the Advantage RT for PCR kit (Clontech, Palo Alto, California). To measure the expression levels of Hsp90 transcripts, quantitative real-time PCR was carried out as follows. Primers were designed manually to amplify approximately 200 bp of Hsp90. Forward primer KM99 (5' CGT TAA GGG TGT TGT AGA CTC 3') was used in combination with reverse primer KM100 (5' TGT CTG GCG AGA TTC TCC AG 3') to amplify a portion of the Hsp90 transcript from *E. acervulina*. Forward primer KM81 (5' GTG AAG GGT GTT GTT GAC TC 3') was used in combination with reverse primer KM82 (5' AGT CTG ACG AGA CTC TCC AG 3') to amplify a portion of the Hsp90 transcript from *E. tenella*. A fragment of the *E. tenella* small subunit ribosomal RNA (SSU rRNA) was used as a control. Each reaction was carried out in triplicate using the Brilliant SYBR Green kit (Stratagene, La Jolla, California). The Mx3000p system (Stratagene) was used in generating and detecting fluorescently labeled products. The expression of each transcript was normalized to SSU rRNA using the Q-gene program (Muller et al., 2002). The thermocycling conditions were as follows. Initial heat activation of polymerase, 95 C for 7 min; denaturation, 94 C for 30 sec; annealing, 65 C for 30 sec; extension, 72 C for 1 min; and a final extension of 5 min at 72 C. Cycles 2–4 were repeated 47 times.

Cloning and sequencing

All PCR products were cloned into the pCR2.1 vector using the TA Cloning Kit (Invitrogen) following the manufacturer's recommended protocol. Inserts were sequenced using vector-specific primers M13 forward and M13 reverse. All sequencing reactions were performed using the Big Dye 3.1 sequencing kits (Applied Biosystems, Foster City, California) with nonisotopic dye terminators and analyzed on an automated sequencer (Perkin Elmer ABI Prism 377 DNA Sequencer, Applied Biosystems, Foster City, California). Sequences obtained were compared with those in GenBank using the BLAST algorithm (Altschul et al., 1990). Chromatograms were viewed and edited using the Sequencer 4.1 program (Gene Codes Corp., Ann Arbor, Michigan). Nucleotide sequence data reported in this study are available in the GenBank®, EMBL, and DDBJ databases under the accession number AY459429.

Sequence and evolutionary analyses

Amino acid alignments were constructed using the ClustalX software (Thompson et al., 1997) with minor manual corrections. Nucleotide sequences were aligned and gapped manually based on protein alignments to retain codon positions. Phylogenetic trees were reconstructed from these nucleotide alignments using maximum likelihood (Felsenstein, 1981). To implement a model of evolution which best fits our data, likelihood scores for 56 different models of evolution were calculated in PAUP* (Swofford, 1998). The scores were then compared using the hierarchical likelihood ratio test (Huelsenbeck and Crandall, 1997) using Modeltest, version 3.04 software (Posada and Crandall, 1998), and the selected model of evolution (GTR + G + I) was used in the analysis. This model of evolution was chosen because it was significantly more likely to produce trees with higher log likelihood values than other simpler models tested. The *Escherichia coli* Hsp90 homolog (hspG) was used as an outgroup in phylogenetic reconstruction.

RESULTS

Isolation and characterization of a full-length cDNA encoding *Eimeria acervulina* Hsp90

While screening random ESTs from a library constructed from cDNA synthesized from IELs of chickens infected with *E. acervulina* and *E. maxima* (Min et al., 2003; Min and Lil-lehoj, 2004), a single clone was identified, which contained significant sequence identity with Hsp90 of *E. tenella* (GenBank AF042329). The sequence of this clone was obtained by entirely sequencing the clone from both DNA strands. Because a single cDNA library was made from birds infected with 2 *Eimeria* spp., it could not be determined whether this clone was derived from *E. acervulina* or *E. maxima*. To determine the origin of this clone, 2 sets of oligonucleotides were designed based on its sequence and used in PCR with genomic DNA of *E. acervulina* or *E. maxima* as template. Only in reactions using *E. acervulina* genomic DNA as target were products found to contain Hsp90 sequence exactly matching those found in the cDNA clone. To rule out that the negative results in reactions using *E. maxima* genomic DNA were not due to inappropriate PCR conditions, gradient PCR was carried out. In no case did *E. maxima* support amplification of products at these parameters (data not shown). Because we were only able to amplify Hsp90 from genomic DNA of *E. acervulina*, and every attempt to isolate this gene from *E. maxima* failed, we believe that the full-length cDNA clone contains the *E. acervulina* homolog of Hsp90. In entirety, this clone is composed of 2,653 bp, and contains a single open reading frame of 2,139 bp. Also included, is a 133-bp 5' untranslated region (UTR) and a 381-bp 3' UTR (data not shown). The amino acid translation of *E. acervulina* Hsp90 is shown aligned with Hsp90 sequences from oth-

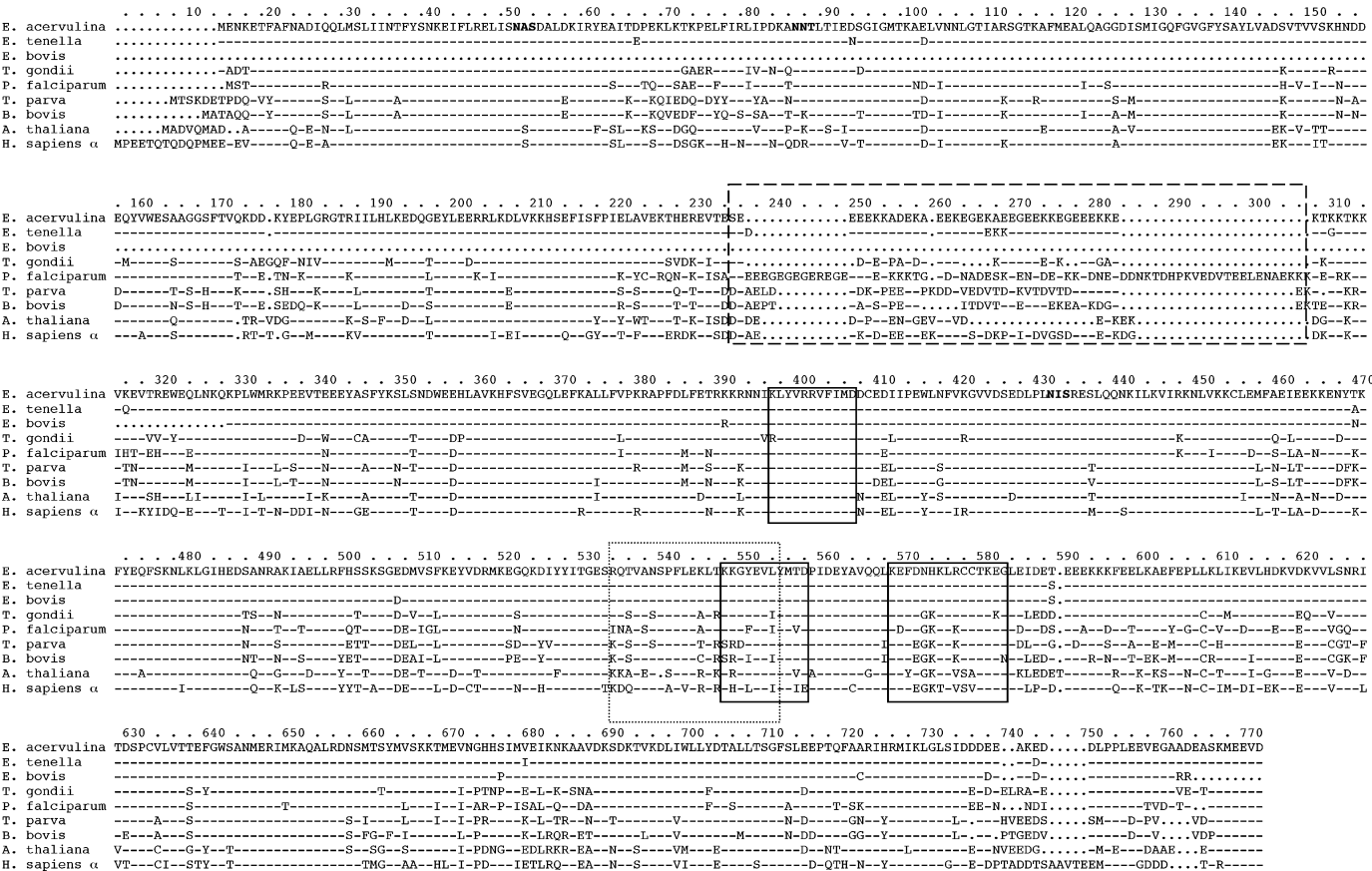


FIGURE 1. Alignment of the amino acid sequences of *Eimeria acervulina* Hsp90 with Hsp90 of *Eimeria tenella* (AF042329), *Eimeria bovis* (U45449), *Toxoplasma gondii* (AY292370), *Plasmodium falciparum* (Z229667), *Theileria parva* (M57386), *B. bovis* (AF136649), *A. thaliana* (Y07613), and *Homo sapiens alpha* (NM.005348). These sequences were used in the analysis throughout the entire study. Dashes denote sequence identity, whereas dots denote gaps. The highly variable acidic domain is boxed with dashed lines, the potential ATP-binding sites are boxed with solid lines, and the conserved domain, which may play a role in calmodulin binding, is boxed with dotted lines. The 3 potential N-glycosylation sites are highlighted in bold type.

er apicomplexans as well as *Arabidopsis thaliana* and *Homo sapiens* in Figure 1. The *E. acervulina* Hsp90 shares 98.3 and 97.7% amino acid identity with Hsp90 of *E. tenella* and partial Hsp90 sequence of *E. bovis*, respectively. At the nucleotide level, *E. acervulina* Hsp90 shares 84.9 and 79.0% sequence identity with Hsp90 of *E. tenella* and partial Hsp90 sequence of *E. bovis*, respectively. Although the greatest sequence identity is found between species of *Eimeria*, Hsp90 homologs exhibit considerable sequence conservation throughout the Apicomplexa (Table I).

TABLE I. Percent nucleotide (NT) and amino acid (AA) identity of Hsp90 among the Apicomplexa.

	<i>Eimeria acervulina</i>		<i>E. tenella</i>		<i>E. bovis</i>	
	NT	AA	NT	AA	NT	AA
<i>E. acervulina</i>	100	100	84.9	98.3	79.7	97.7
<i>E. tenella</i>	84.9	98.3	100	100	79.0	97.9
<i>E. bovis</i>	79.7	97.7	79.0	97.9	100	100
<i>Toxoplasma gondii</i>	74.7	84.5	76.7	83.8	69.9	84.4
<i>Plasmodium falciparum</i>	66.3	78.2	62.9	78.3	68.4	79.6
<i>Theileria parva</i>	67.6	76.0	69.9	75.7	66.0	79.6
<i>B. bovis</i>	68.2	74.4	68.2	74.4	67.0	79.6

a).

Protein	Residues	Sequence															
Type B consensus		H/R/K		X (5–8)					Φ	X	Φ	Φ	D/E				
Human α	395-405	K	L	Y	V	R	R	V	F	I	M	D					
<i>Eimeria</i>	395-405	K	L	Y	V	R	R	V	F	I	M	D					
<i>P. falciparum</i>	395-405	K	L	Y	V	R	R	V	F	I	M	D					
Human α	546-556	K	H	G	L	E	V	I	Y	M	I	E					
<i>Eimeria</i>	546-556	K	K	G	Y	E	V	L	Y	M	T	D					
<i>P. falciparum</i>	546-556	K	K	G	F	E	V	I	Y	M	V	D					
Type A consensus		A/G	X	X	X	X	G	K	T/S	X	X	X	X	X	X	I/V	
Human α	567-581	L	K	E	F	E	G	K	T	L	V	S	V	T	K	E	G
<i>Eimeria</i>	567-581	L	K	E	F	D	N	H	K	L	R	C	C	T	K	E	G
<i>P. falciparum</i>	567-581	L	K	D	F	D	G	K	K	L	K	C	C	T	K	E	G

b).

Protein	Residues	Sequence
Human α	532-552	K D Q V A N S A F V E R L R K H G L E V I
<i>Eimeria</i>	532-552	R Q T V A N S P F L E K L T K K G Y E V I
<i>P. falciparum</i>	532-552	I N A V S N S P F L E A L T K K G F E V I

FIGURE 2. (a). Conservation of putative ATP-binding sites and (b) conservation of putative calmodulin-binding sites between Hsp90 of *Eimeria* species, *Plasmodium falciparum*, and *Homo sapiens* α. Amino acid positions are relative to Figure 1. Φ stands for hydrophobic amino acid and X for any amino acid. Identical and conservative changes with respect to consensus sequence are in bold type.

endoplasmic reticulum (ER) (Lindquist and Craig, 1988). *Eimeria* spp. Hsp90 contains the consensus sequence EEVD at its C terminus, which is conserved in cytosolic homologs of Hsp90 (Lindquist and Craig, 1988) (Fig. 1). The larger organelle Hsp90 contains the ER retention signal KDEL at the C terminus (Pelham, 1988). An ER retention sequence is not found in *Eimeria* spp. Hsp90 described in this study. Its predicted size corresponds to other cytosolic Hsp90 proteins; therefore, the sequences presented in this study most likely encode cytosolic Hsp90.

It has been previously reported that Hsp90 proteins possess adenosine triphosphate (ATP)-binding sites and are capable of autophosphorylation (Csermely and Kahn, 1991). Consensus ATP-binding sites have also been located in Hsp90 of *Eimeria* species (Fig. 2a). Two putative type-B ATP-binding sites (Chin et al., 1988) were found in all 3 *Eimeria* species for which Hsp90 sequence is available. The conservation of this consensus sequence has also been previously reported in Hsp90 of *P. falciparum* (Bonney et al., 1994). The conservation of type-A ATP-binding sites (Chin et al., 1988) has been reported in mammalian Hsp90 (Csermely and Kahn, 1991). However, this site is not conserved in any of the *Eimeria* sequences (Fig. 2a). All Hsp90 sequences contain a highly variable domain that is characterized by the presence of acidic amino acid residues. This domain is also found in *Eimeria* spp. Hsp90 and is boxed by dashed lines in Figure 1. In this highly acidic region, Bonney et al. (1994) found an area of homology between calre-

ticulin, the major calcium-binding protein of the ER, and *P. falciparum* Hsp90. This homology is not found in Hsp90 of *Eimeria* species because the acidic region found in these species is significantly shorter than that of *P. falciparum*. Mammalian Hsp90 proteins contain a calmodulin-binding site (Minami et al., 1993), which spans 21 residues at positions 532–552 and overlaps with 1 of the type-B ATP-binding sites (Fig. 1). In this region, *Eimeria* spp. Hsp90 contains significant sequence identity with human Hsp90 α (Fig. 2b), with 16 of the 21 residues being identical or conserved. In the same region, the *P. falciparum* sequence contains less homology, with only 13 of the 21 residues being conserved.

Expression of Hsp90 transcripts during *Eimeria* spp. life cycle

To determine the expression levels of Hsp90 transcripts in both *E. tenella* and *E. acervulina*, cDNA synthesized from 4 different developmental stages, i.e., unsporulated oocysts, sporulated oocysts, sporozoites, and merozoites were tested using standard reverse transcription-polymerase chain reaction (RT-PCR). On examination of amplified products, it was apparent that Hsp90 transcripts were readily detectable at all stages of *E. tenella* development. Although transcripts were detected in *E. acervulina* unsporulated oocysts, sporozoites, and merozoites, no transcript was present in reactions carried out using cDNA generated from sporulated oocysts of this species (data

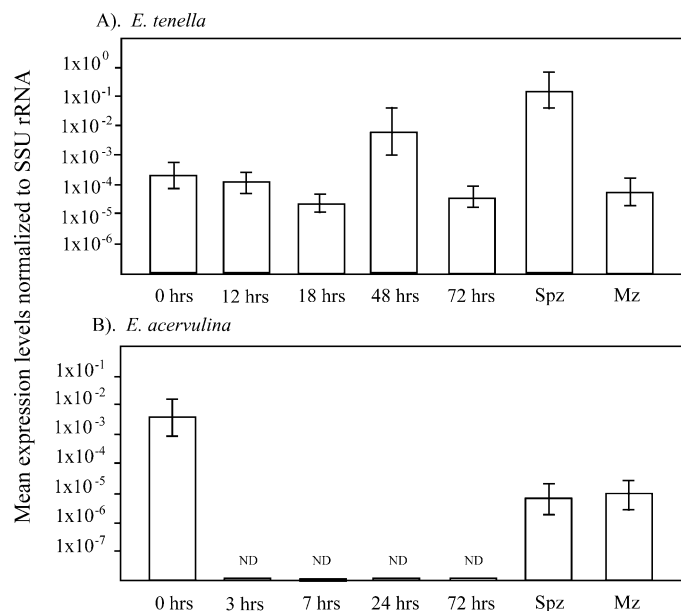


FIGURE 3. Analysis of expression of the Hsp90 gene in (A) *Eimeria tenella* and (B) *Eimeria acervulina* using quantitative real-time RT-PCR. Spz, sporozoites; Mz, merozoites; ND, no transcripts detected.

not shown). To further investigate this unusual difference in expression profiles, additional cDNA samples were obtained that included 5 time-points during oocyst sporulation from both *E. tenella* and *E. acervulina*. These time-points were sampled throughout oocyst development, ranging between 0 (unsporulated oocysts) and 72 hr (fully sporulated oocysts). Because *E. acervulina* oocysts sporulate more rapidly than *E. tenella* (Norton and Chard, 1983), more time-points were sampled early on during treatment, i.e., 0, 3, 7, 24, and 72 hr, whereas the time-points for *E. tenella* were spread out more evenly over the course of 72 hr, i.e., 0, 12, 18, 48, and 72 hr. cDNAs from both species representing the various stages of sporulating oocysts, as well as sporozoites and merozoites were then subjected to the highly sensitive technique of quantitative RT-PCR using real-time methods. The results of these experiments are shown in Figure 3. It is apparent that although *E. tenella* Hsp90 transcripts are present at all stages tested (Fig. 3a), with the highest level of transcripts observed in sporozoites, the expression profile of *E. acervulina* Hsp90 differs drastically (Fig. 3b). These data indicate that *E. acervulina* transcripts are expressed at high levels by unsporulated oocysts (0 hr); however, by 3 hr into the sporulation process the transcripts fall to undetectable levels. Hsp90 transcripts remain undetectable throughout sporulation, with expression levels being once again detectable in cDNA of sporozoites and merozoites.

Hsp90 evolution

A phylogenetic analysis was carried out to determine the evolutionary relationships of *Eimeria* spp. Hsp90. To include the partial sequence from *E. bovis* in the analysis, only the nucleotides that overlap with the *E. bovis* gene fragment were used. This included nucleotides immediately 3' of the regions encoding the highly variable acidic domains (see Fig. 1) to the stop codon. Using the maximum likelihood criterion, a single tree

was obtained (Fig. 4). The Hsp90 sequences from *E. acervulina* and *E. tenella* were included in a monophyletic coccidia-specific clade, which included sequence from *E. bovis* and *T. gondii*, with *T. gondii* sequences being basal to all *Eimeria* spp. sequences. Basal to the coccidia clade were sequences from other apicomplexans, i.e., *P. falciparum*, *T. parva*, and *B. bovis*. Hsp90 of the kinetoplastids formed a sister clade to the apicomplexans. Sequences of organelle Hsp90 have been previously isolated from 3 species of apicomplexans, i.e., *P. falciparum*, *Plasmodium yoelii yoelii*, and *C. parvum*. These 3 sequences form a clade distinct and basal to all the sequences from cytosolic versions of Hsp90.

DISCUSSION

Hsp90 represents 1 of the most conserved proteins and is found in members of all evolutionary lineages. In this study, we describe a full-length cDNA encoding the *E. acervulina* Hsp90 homolog. This transcript was isolated from epithelial tissues of infected chickens and was only 1 of 5 clones of *Eimeria* sp. origin isolated from random screening of over 30,000 ESTs (data not shown). Complete analysis of 1 of these clones suggests that Hsp90 is expressed during infection; therefore, the potential use of *Eimeria* spp. Hsp90 as an antigen in subunit vaccines and its immunostimulatory capabilities should be investigated.

Hsp90 proteins can bind ATP and autophosphorylate serine residues (Csermely and Kahn, 1991). Two types of ATP-binding sites are conserved in mammalian Hsp90. The 2 type B-binding sites found in *Eimeria* spp. Hsp90 are well conserved; however, the conservation of the single type A site is low (Fig. 2a). The lack of conservation of the type A site may lower binding affinity for ATP, but only biochemical studies will be able to resolve whether the lack of this site has any effect on ATP binding in *Eimeria* spp. Hsp90. A calmodulin-binding domain is also present in Hsp90 of *Eimeria* spp. This domain in *Eimeria* spp. Hsp90 shows a higher degree of homology to the human consensus sequence than that of *P. falciparum* (Fig. 2b).

Although Hsp90 of *E. tenella* and *E. acervulina* are quite similar in sequence, the expression profiles of these 2 genes differ drastically. We found that *E. tenella* Hsp90 is transcribed constitutively in all developmental stages tested (although the levels of transcripts differ between stages), whereas *E. acervulina* Hsp90 is not transcribed by developing or fully sporulated oocysts. The observed expression patterns were obtained using several different batches of parasites in conjunction with standard RT-PCR methods. These results were reproduced using quantitative methods; therefore, we believe that differences in expression profiles between these 2 species are bona fide and not an artifact. Clark et al. (1996) reported that Hsp90 transcripts in *E. bovis* Hsp90 were present in fully sporulated oocysts and merozoites; however, transcripts were undetectable in unsporulated or partially sporulated oocysts. Altogether, these results indicate that Hsp90 transcription in *E. bovis* and *Eimeria* spp. of poultry is regulated differently during parasite development. The constitutive expression of Hsp90 in *E. tenella* matches the expression profiles of most other eukaryotes in which Hsp90 has been studied. The significance of transcription differences between *Eimeria* species is, at this point, unknown, and expression analyses at the translational level will be nec-

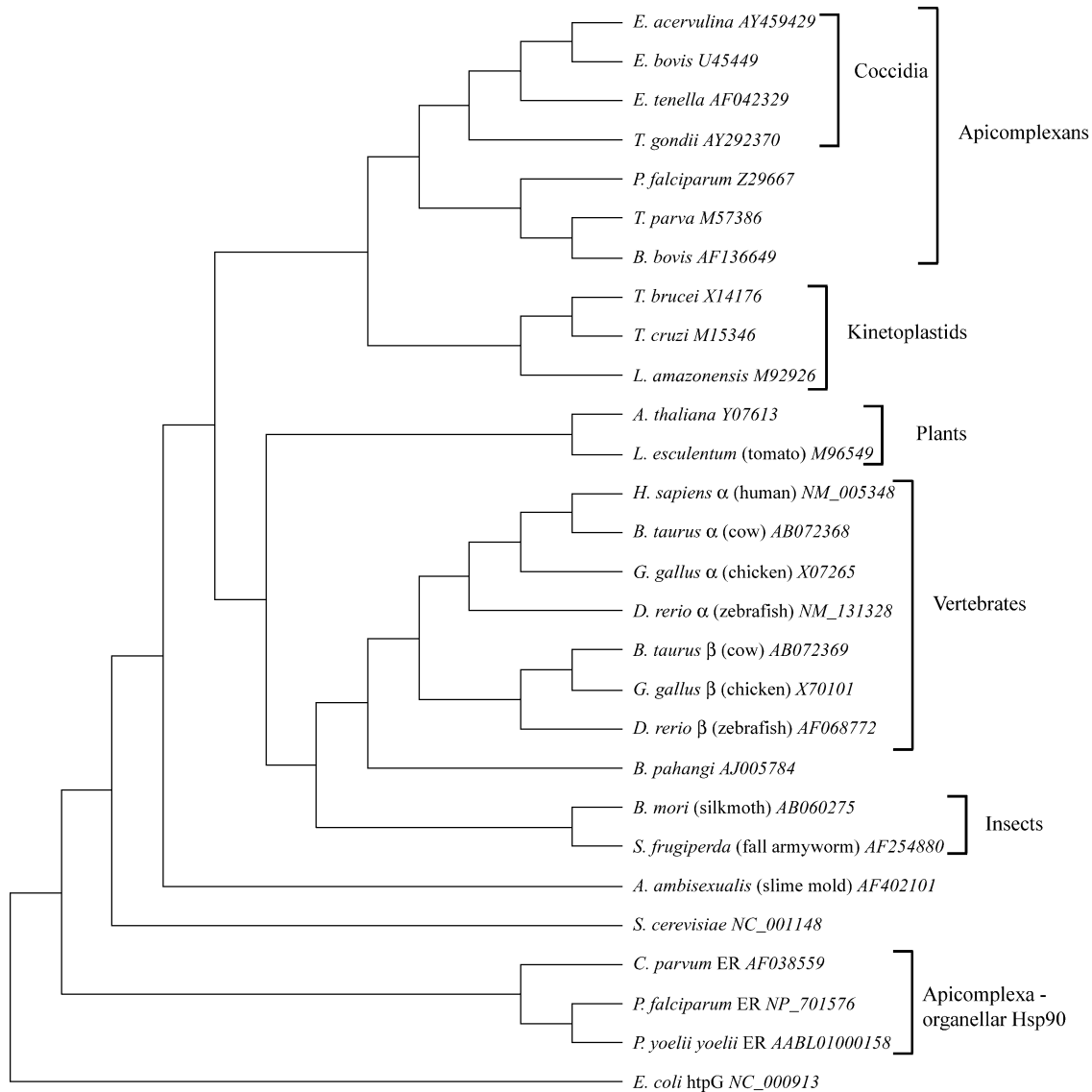


FIGURE 4. Phylogenetic tree reconstructed using maximum likelihood using sequences of Hsp90 genes from 28 representative taxa.

essary to determine whether differential expression during transcription ultimately affects protein levels in these parasites.

On the basis of sequence identity and presence of conserved residues, it is most likely that the Hsp90 sequences analyzed in this study encode the cytosolic versions of Hsp90. Evolutionary analysis further corroborates this finding because the sequences from organellar Hsp90 of other apicomplexans form a distinct clade basal to all cytosolic Hsp90 sequences. This suggests that the gene duplication event that resulted in formation of cellular and organellar copies of Hsp90 occurred very early during eukaryotic evolution. A similar conclusion was drawn from analysis of organelle and cytosolic Hsp90 sequences from higher eukaryotes and plants (Gupta, 1995). Other gene duplications have also been observed in the Hsp90 family, the most documented of which occurred early in vertebrate evolution, producing 2 functional copies of the cytosolic Hsp90 (α and β) (Gupta, 1995). Through Southern analysis, using *E. bovis* Hsp90 as a probe, Clark et al. (1996) concluded that *E. acer-*

vulina and *E. bovis* Hsp90 also exist as single-copy genes. All genomic analyses carried out thus far indicate that the *Eimeria* spp. Hsp90 locus is stable and no duplications have occurred at this locus during the evolution of these species.

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